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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/618,443	07/11/2003	James Linder	38-7011642001 (11.023011)	4868

38732 7590 04/27/2006

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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1637	

DATE MAILED: 04/27/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/618,443	LINDER ET AL.	
	Examiner	Art Unit	
	Stephanie K. Mummert	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37* is/are pending in the application.

4a) Of the above claim(s) *30-35* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-29,36 and 37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *1/14/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election without traverse of Group I in the reply filed on March 15, 2006 is acknowledged.

2. Claims 30-35 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on March 15, 2006.

3. Claims 1-29 and 36-37 are pending and will be examined.

Information Disclosure Statement

4. The information disclosure statement (IDS) submitted on January 14, 2004 was filed in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Objections

5. Claims 24-25 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. As currently recited, claims 24 and 25 reiterate a limitation which is recited in claim 1. Within claim 1, the sensor is conjugated to a chromophore, light is applied and the emission of light is measured. Within claims 24 and 25, the sensor is conjugated to a detectable moiety, a limitation which is redundant and does not further limit the subject matter of the claim as the sensor is labeled with a detectable moiety and the label is detected within claim 1.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

7. Claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 are rejected under 35 U.S.C. 102(b) as being anticipated by Bresser et al. (US Patent 5,225,326; July 1993). Bresser teach a one-step in situ hybridization assay for detecting multiple biopolymers in the same cell (Abstract).

With regard to claim 1, Bresser teach a method comprising:

a) providing a sample that is suspected of containing a target (col. 12, lines 13-20, where samples are broken apart and cells are fixed and processed; examples 3-12);

b) providing a sensor that can bind to the target in an alcoholic preservative solution, said sensor conjugated to a chromophore (Example 9, col. 23-24, lines 43-46, where an RNA probe directly labeled with fluorescein is added to the hybridization reaction; Example 6, col. 21, lines 22-39, where human breast tissue was fixed and hybridized for 20 minutes by incubation at 55°C with a one step cocktail of 20% ethanol, 30% formamide and other components including an RNA probe labeled with Pontamine Sky Blue™);

c) contacting the sample with the sensor in the alcoholic preservative solution under conditions in which the sensor can bind to the target, if present (Example 9, col. 23-24, lines 38-64, where

cell suspension was fixed in 35% ethanol, 55% formamide, 5% formalin and other components along with an anti-sense RNA probe directly labeled with fluorescein and hybrid detection follows; Example 6, col. 21, lines 22-39, where human breast tissue was fixed and hybridized for 20 minutes by incubation at 55°C with a one step cocktail of 20% ethanol, 30% formamide and other components including an RNA probe labeled with Pontamine Sky Blue™);

d) applying a light source to the solution that can excite the chromophore (Example 9, col. 24, lines 59-68, where fluorescence emitted from each cell is visualized and recorded through photomicroscopy using a Nikon Photophot fluorescence microscope; Example 6, col. 21, lines 40-46, where labeled probes were detected by photography with a microscope with fluorescent capabilities); and

e) detecting whether light is emitted from the target (Example 9, col. 24, lines 59-68, where fluorescence emitted from each cell is visualized and recorded through photomicroscopy using a Nikon Photophot fluorescence microscope; Example 6, col. 21, lines 40-46, where labeled probes were detected by photography with a microscope with fluorescent capabilities).

With regard to claim 2, Bresser teach an embodiment of claim 1, wherein the sample is selected from the group consisting of a blood (example 5), a section of tissue (example 6), or in vitro cell culture constituent (examples 2-4 and 8-10, where various cell lines provide the target sample).

With regard to claims 3 and 4, Bresser teach an embodiment of claim 1, wherein the sensor comprises an aptamer and a polynucleotide (Example 9, col. 23-24, lines 43-46, where an RNA probe directly labeled with fluorescein is added to the hybridization reaction and wherein an RNA probe meets the limitation of aptamer and polynucleotide).

With regard to claim 8, Bresser teach an embodiment of claim 1, wherein the chromophore is a fluorophore (Example 9, col. 23-24, lines 43-46, where an RNA probe directly labeled with fluoroscein is added to the hybridization reaction, wherein fluorescein is a fluorophore).

With regard to claims 9 and 11, Bresser teach an embodiment of claims 8 and 9, wherein the fluorophore is a fluorescent dye (Example 9, col. 23-24, lines 43-46, where an RNA probe directly labeled with fluoroscein is added to the hybridization reaction, wherein fluorescein is a fluorophore).

With regard to claim 12, Bresser teach an embodiment of claim 11, wherein the fluorescent dye is fluorescein (Example 9, col. 23-24, lines 43-46, where an RNA probe directly labeled with fluoroscein is added to the hybridization reaction, wherein fluorescein is a fluorophore).

With regard to claim 14, Bresser teach an embodiment of claim 1, wherein the target is DNA (col. 3, lines 17-23, where the probe may be targeted to cellular RNA or cellular DNA).

With regard to claim 15, Bresser teach an embodiment of claim 1, wherein the target is RNA (Example 9, where the target was an mRNA target within the cells and Example 6, where the targets were particular oncogene mRNA targets).

With regard to claim 16, Bresser teach an embodiment of claim 1, wherein the sample is a cellular fraction (Example 9, where K562 cells were grown in cell culture, pelleted and processed).

With regard to claim 18, Bresser teach an embodiment of claim 1, wherein said target is a pathological organism or component or product thereof (Examples 7 and 10-11, where the target was HIV sequence within cells).

With regard to claim 19, Bresser teach an embodiment of claim 1, wherein the target is a virus or component or product thereof (Examples 7 and 10-11, where the target was HIV sequence within cells).

With regard to claim 20, Bresser teach an embodiment of claim 1, further comprising comparing a result from said detecting to a result obtained from a control sample (Example 6, col. 21, lines 47-57, where a variety of controls were included, see Figure 5; c-myc probe was used as a positive control, c-sis probe was used as a negative control; SIS/PDGF-B expression was also used to demonstrate expression in the epithelial components of the breast carcinoma).

With regard to claim 21, Bresser teach an embodiment of claim 20, where the control sample is a positive control (Example 6, col. 21, lines 47-57, where a variety of controls were included, see Figure 5; c-myc probe was used as a positive control).

With regard to claim 22, Bresser teach an embodiment of claim 20, where the control sample is a negative control (Example 6, col. 21, lines 47-57, where a variety of controls were included, see Figure 5, c-sis probe was used as a negative control).

With regard to claim 23, Bresser teach an embodiment of claim 1, further comprising washing said sample prior to said detecting (col. 13, line 64 to col. 14, line 8, where unbound probe is washed away to wash away noise).

With regard to claim 24 and 25, Bresser teach an embodiment of claim 1, wherein the sensor is conjugated to a detectable moiety and where the sensor is itself detectable (Example 9,

col. 23-24, lines 43-46, where an RNA probe directly labeled with fluoroscein is added to the hybridization reaction; col. 24, lines 59-68, where fluorescence emitted from each cell is visualized and recorded through photomicroscopy).

With regard to claim 26, Bresser teach an embodiment of claim 1, wherein the method is automated (col. 14, lines 14-19, where results are visually analyzed using an automated system).

With regard to claim 27, Bresser teach an embodiment of claim 1, wherein the method is performed manually (col. 14, lines 11-13, where results are visualized manually on a fluorescent microscope).

With regard to claim 28, Bresser teach a method for identifying a sensor which specifically binds to a desired target, comprising:

a) contacting a sample suspected of containing a target of interest with a detectable sensor, wherein said contacting takes place in a preservative solution comprising an amount of one or more water soluble alcohols effective to preserve such solution against at least one contaminant (Example 9, col. 23-24, lines 38-64, where cell suspension was fixed in 35% ethanol, 55% formamide, 5% formalin and other components along with an anti-sense RNA probe directly labeled with fluorescein and hybrid detection follows; Example 6, col. 21, lines 22-39, where human breast tissue was fixed and hybridized for 20 minutes by incubation at 55°C with a one step cocktail of 20% ethanol, 30% formamide and other components including an RNA probe labeled with Pontamine Sky Blue™); and

b) detecting whether said sensor has bound to said target (Example 9, col. 24, lines 59-68, where fluorescence emitted from each cell is visualized and recorded through photomicroscopy using a

Nikon Photophot fluorescence microscope; Example 6, col. 21, lines 40-46, where labeled probes were detected by photography with a microscope with fluorescent capabilities).

8. Claim 28 is rejected under 35 U.S.C. 102(e) as being anticipated by Lorincz et al. (US Patent 6,969,585; November 2005). Lorincz teaches a universal collection medium for cell collection which allows for cytology and direct molecular analysis on cells preserved in a single sample (Abstract).

With regard to claim 28, Lorincz teach a method for identifying a sensor which specifically binds to a desired target, comprising:

a) contacting a sample suspected of containing a target of interest with a detectable sensor, wherein said contacting takes place in a preservative solution comprising an amount of one or more water soluble alcohols effective to preserve such solution against at least one contaminant (Example 1, where the DNA/RNA protocol was taught, where a DNA biotinylated probe was added directly to samples and incubated at 65°C for hybridization, followed by transfer to a streptavidin coated microplate, followed by chemiluminescent detection; col. 10, where the formulation of the universal collection medium are listed, which comprise an alcoholic preservative solution; see Examples 3-5, where samples were incubated and hybridized in UCM formulations indicated at col. 10); and

b) detecting whether said sensor has bound to said target (Example 1, col. 10, lines 1-5, where the plate was read in a luminometer and data was expressed as signal-to-noise).

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. Claims 1-4, 14-16, 18-19 and 23-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lorincz et al. (US Patent 6,969,585; November 2005) as applies to claim 28 above and further in view of Shah et al. (US Patent 6,165,723; December 2000). Lorincz teaches a universal collection medium for cell collection which allows for cytology and direct molecular analysis on cells preserved in a single sample (Abstract).

With regard to claim 1, Lorincz teach a method comprising:

a) providing a sample that is suspected of containing a target (example 1, col. 10, where the protocol of detecting a nucleic acid is taught; example 3-5, the samples were HPV 16 positive cancer cells, CaSki were placed in the universal collection medium and tested for HPV DNA or RNA targets);

b) providing a sensor that can bind to the target in an alcoholic preservative solution, said sensor conjugated to a chromophore (Example 1, where the DNA/RNA protocol was taught, where a DNA biotinylated probe was added directly to samples and incubated at 65°C for hybridization, followed by transfer to a streptavidin coated microplate, followed by chemiluminescent detection; col. 10, where the formulation of the universal collection medium are listed, which comprise an alcoholic preservative solution; see Examples 3-5, where samples were incubated and hybridized in UCM formulations indicated at col. 10);

c) contacting the sample with the sensor in the alcoholic preservative solution under conditions in which the sensor can bind to the target, if present (Example 1, where the DNA/RNA protocol was taught, where a DNA biotinylated probe was added directly to samples and incubated at 65°C for hybridization, followed by transfer to a streptavidin coated microplate, followed by chemiluminescent detection); and

e) detecting the target (Example 1, col. 10, lines 1-5, where the plate was read in a luminometer and data was expressed as signal-to-noise).

With regard to claim 2, Lorincz teach an embodiment of claim 1, wherein the sample is selected from the group consisting of a urine, a vaginal swab, a pap smear, a needle biopsy and a section of tissue (col. 7, lines 46-57, where multiple methods of obtaining cells are noted, including those listed).

With regard to claims 3 and 4, Lorincz teach an embodiment of claim 1, wherein the sensor comprises an aptamer and a polynucleotide (col. 2, lines 59-61, where DNA, RNA, protein or carbohydrates are measured; col. 9, lines 32-39).

With regard to claim 14, Lorincz teach an embodiment of claim 1, wherein the target is DNA (col. 2, lines 59-61, where DNA, RNA, protein or carbohydrates are measured; col. 9, lines 32-39).

With regard to claim 15, Lorincz teach an embodiment of claim 1, wherein the target is RNA (col. 2, lines 59-61, where DNA, RNA, protein or carbohydrates are measured; col. 9, lines 32-39).

With regard to claim 16, Lorincz teach an embodiment of claim 1, wherein the sample is a cellular fraction (Example 3-5, where HPV 16 positive cancer cells (CaSki) were placed in UCM and other fixatives and where the cells were studied at a concentration of $0.8x\ 10^6$ cells).

With regard to claim 18, Lorincz teach an embodiment of claim 1, wherein said target is a pathological organism or component or product thereof (Examples 3-5, where the target is HPV).

With regard to claim 19, Lorincz teach an embodiment of claim 1, wherein the target is a virus or component or product thereof (Examples 3-5, where the target is HPV).

With regard to claim 23, Lorincz teach an embodiment of claim 1, further comprising washing said sample prior to said detecting (Example 1, col. 10, lines 64-66, where wells were washed 6x after hybridization and prior to detection).

With regard to claim 24 and 25, Lorincz teach an embodiment of claim 1, wherein the sensor is conjugated to a detectable moiety and where the sensor is itself detectable (Example 1, where the DNA/RNA protocol was taught, where a DNA biotinylated probe was added directly to samples and incubated at 65°C for hybridization, followed by transfer to a streptavidin coated microplate, followed by chemiluminescent detection).

With regard to claim 26, Lorincz teach an embodiment of claim 1, wherein the method is automated (col. 9, lines 46-67, where the method can be carried out using devices adapted to the method).

With regard to claim 27, Lorincz teach an embodiment of claim 1, wherein the method is performed manually (col. 9, lines 46-67, where in the converse situation, the method can be carried out without using devices adapted to the method).

Regarding claim 1, Lorincz teaches the method of binding a sensor to a target in an alcohol containing solution, however Lorincz does not teach the steps directed to d) applying a light source to the solution that can excite the chromophore and e) detecting whether light is emitted from the target.

With regard to claim 8, Shah teach an embodiment of claim 1, wherein the chromophore is a fluorophore (col. 9, lines 5-30, where a fluorescein labeled probe complementary to B. microti was hybridized to fixed cells).

With regard to claims 9 and 11, Shah teach an embodiment of claims 8 and 9, wherein the fluorophore is a fluorescent dye (col. 9, lines 5-30, where a fluorescein labeled probe complementary to B. microti was hybridized to fixed cells).

With regard to claim 12, Shah teach an embodiment of claim 11, wherein the fluorescent dye is fluorescein (col. 9, lines 5-30, where a fluorescein labeled probe complementary to B. microti was hybridized to fixed cells).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe taught by Lorincz to substitute a direct chromophore label as taught by Shah for the biotin label currently disclosed. As noted by Shah, "The sample is then rinsed with Evans Blue to counter stain the host cell, so as to see clearly fluorescent labeled probe(s) bound to specific nucleic acids of the pathogens which may be present within the sample (e.g., within the cells of the sample)" (col. 2, lines 59-63). Furthermore, as noted by Shah, "This procedure allows for the use of non-radiolabeled probes, which have a much longer shelf life and do not require special storage space. Either direct detection system using dark field microscopy can be used" (col. 6, lines 1-4). Therefore, one of

ordinary skill in the art at the time the invention was made would have been motivated to substitute the direct fluorescent label taught by Shah for the biotin label taught by Lorincz with a reasonable expectation for success.

11. Claim 5, 7, 29 and 36-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bresser et al. (US Patent 5,225,326; July 1993) as applied to claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 above, and further in view of Hyldig-Nielsen et al. (US Patent 6,280,946; August 2001). Bresser teach a one-step in situ hybridization assay for detecting multiple biopolymers in the same cell (Abstract).

Bresser teaches the limitations of claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 as recited in the 102 rejection stated above. However, Bresser does not teach the inclusion of a probe which comprises peptide nucleic acid (PNA) or the inclusion of a plurality of probes each of which comprises a corresponding detectable label.

Hyldig-Nielsen teaches the inclusion of a peptide nucleic acid probe into a method of multiplex-FISH analysis (Abstract).

With regard to claim 5, Hyldig-Nielsen teach an embodiment of claim 1, wherein the sensor comprises a peptide nucleic acid (Abstract, col. 10, lines 58-66, where PNA probes represent a preferred embodiment of the invention).

With regard to claim 7, Hyldig-Nielsen teach an embodiment of claim 1, wherein the sample is contacted with a plurality of different sensors, each of said plurality comprising a corresponding detectable label, wherein each of said plurality can selectively bind to a corresponding different target (col. 7, lines 58-63, where the invention is directed towards

multiplex analysis; col. 15, lines 60-63, where each individual probe is independently detectable).

With regard to claim 29, Hyldig-Nielsen teach an embodiment of claim 28, wherein the method is performed on a plurality of candidate sensors (col. 7, lines 58-63, where the invention is directed towards multiplex analysis; col. 15, lines 60-63, where each individual probe is independently detectable).

With regard to claim 36, Hyldig-Nielsen teach an embodiment of claim 1, wherein said target is a bacterium or component or product thereof (Table 2, where PNA probes are directed to detect bacteria; Example 12, col. 28, lines 26-43, where a culture of *E. coli* is examined using the multiplex-PNA-FISH method disclosed).

With regard to claim 37, Hyldig-Nielsen teach an embodiment of claim 1, wherein said target is a yeast or component or product thereof (Table 2, where PNA probes are directed to detect eucarya; Example 12, col. 28, lines 44-65, where a culture of *Saccharomyces cerevisiae* is examined using the multiplex-PNA-FISH method disclosed).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the PNA probe and the plurality of probes taught by Hyldig-Nielsen into the method of in situ hybridization taught by Bresser. As noted by Hyldig-Nielsen, the inclusion of PNA probes specifically provides that "Because the PNA probe can efficiently and preferably, hybridize to nucleic acid under these conditions of low salt, the PNA probes can be designed to target rRNA which cannot be targeted by traditional nucleic acid probes". Hyldig-Nielsen goes on to note that "PNA probes of this invention typically generate stronger signals than can be achieved with nucleic acid probes of comparable nucleobase sequence" (col.

14, lines 30-45). Furthermore, Hyldig-Nielsen teaches that, "the ability to differentiate between and/or quantitate each of the independently detectable moieties provides the means to multiplex a hybridization assay because the data which correlates with the hybridization of each of the distinctly (independently) labeled probe to a particular target sequence can be correlated with the presence, absence or quantity of each organism sought to be detected in the sample. Consequently, the multiplex assays of this invention may be used to simultaneously detect the presence, absence or quantity of two or more organisms in the same sample and in the same assay" (col. 7, line 66 to col. 8 line 9). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to include the PNA probes in a multiplex-FISH method taught by Hyldig-Nielsen in order to achieve the stated benefit of increased signal strength and probes which target regions inaccessible to traditional nucleic acid probes with a reasonable expectation for success.

12. Claim 6 is rejected under 35 U.S.C. 103(a) as being unpatentable over Bresser et al. (US Patent 5,225,326; July 1993) as applied to claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 above, and further in view of Kumar et al. (Bioorganic & Medicinal Chemistry, 1998, vol. 8, p. 2219-2222). Bresser teach a one-step in situ hybridization assay for detecting multiple biopolymers in the same cell (Abstract).

Bresser teaches the limitations of claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 as recited in the 102 rejection stated above. However, Bresser does not teach the inclusion of a probe which comprises locked nucleic acid.

Kumar teaches the improvement of duplex stability provided by the inclusion of a locked nucleic acid probe into a method where recognition of complementary DNA or RNA was necessary (Abstract).

With regard to claim 6, Kumar teach an embodiment of claim 1, wherein the sensor comprises a locked nucleic acid (Abstract, p. 2219, where it is noted that LNA provides stability against 3'-exonucleolytic degradation, efficient automated synthesis, good solubility and improved stability of duplexes with DNA or RNA).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the LNA probe taught by Kumar into the method of in situ hybridization taught by Bresser. As noted by Kumar, the inclusion of LNA probes specifically provides, "unprecedented thermal stabilities of duplexes towards complementary DNA and RNA" , stability towards 3'-exonucleolytic degradation, efficient automated oligomerization and good aqueous solubility (p. 2219). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to include the LNA probe taught by Kumar in order to achieve the stated benefit of improved thermal stability of duplexes formed with either DNA or RNA target molecules.

13. Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Bresser et al. (US Patent 5,225,326; July 1993) as applied to claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 above, and further in view of Bruchez, Jr. et al. (Science, 1998, vol. 281, p. 2013-2016). Bresser teach a one-step in situ hybridization assay for detecting multiple biopolymers in the same cell (Abstract).

Bresser teaches the limitations of claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 as recited in the 102 rejection stated above. However, Bresser does not teach the inclusion of a semiconductor nanocrystal as the detectable label.

Bruchez, Jr. teaches the inclusion of a semiconductor nanocrystal into a method of biological staining and diagnostics (Abstract).

With regard to claim 10, Bruchez, Jr. teach an embodiment of claim 9, wherein the fluorophore is a semiconductor nanocrystal (Abstract, Figure 2).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the semiconductor nanocrystal taught by Bruchez, Jr. into the method of in situ hybridization taught by Bresser. As noted by Bruchez, Jr, the semiconductor nanocrystal label provide the benefit that "many sizes of nanocrystals may therefore be excited with a single wavelength of light, resulting in many emission colors that may be detected simultaneously" (p. 2014, col. 1). Furthermore, Bruchez, Jr. teach that "the tenability of the optical features allows for their use as direct probes or as sensitizers for traditional probes. These nanocrystals also have a long fluorescence lifetime, which can allow for time-gated detection of autofluorescence suppression" (p. 2015, col. 2). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to include the semiconductor nanocrystal taught by Bruchez, Jr. in order to achieve the stated benefit of a label that is excitable by a single wavelength of light yet able to provide multiple colors of emission, particularly for the detection of multiple targets simultaneously.

14. Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Bresser et al. (US Patent 5,225,326; July 1993) as applied to claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 above, and further in view of Ylikoski et al. (US Patent 5,256,535; October 1993). Bresser teach a one-step in situ hybridization assay for detecting multiple biopolymers in the same cell (Abstract).

Bresser teaches the limitations of claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 as recited in the 102 rejection stated above. However, Bresser does not teach the inclusion of a lanthanide chelate as the detectable label.

Ylikoski teaches the inclusion of a lanthanide chelate into a method of hybridization (Abstract).

With regard to claim 13, Ylikoski teach an embodiment of claim 9, wherein the fluorophore is a lanthanide chelate (Abstract; col. 3, lines 44-52; col. 4, lines 17-21).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the lanthanide chelate label taught by Ylikoski into the method of in situ hybridization taught by Bresser. As noted by Ylikoski, the inclusion of a lanthanide chelate label specifically provide that "the intensity of fluorescence emitted from the double stranded nucleic acid is a quantitative measure of the nucleotide sequence to be determined" (col. 4, lines 25-28). Ylikoski goes on to note that "the important feature in the invention is the use of water soluble polymeric compounds as a matrix to which a large number of europium or terbium chelates are covalently coupled. This covalent coupling gives a large amplification of the detectable signal" (col. 4, lines 35-42). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to include the lanthanide chelate label taught by

Ylikoski in order to achieve the stated benefit of increased signal strength with a reasonable expectation for success.

15. Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable over Bresser et al. (US Patent 5,225,326; July 1993) as applied to claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 above, and further in view of Fukasawa et al. (Science, 1996, vol. 271, p. 1744-1747) . Bresser teach a one-step in situ hybridization assay for detecting multiple biopolymers in the same cell (Abstract).

Bresser teaches the limitations of claims 1-4, 8-9, 11-12, 14-16, 18-25 and 27-28 as recited in the 102 rejection stated above. However, Bresser does not teach the examination of a centrosomal target.

Fukusawa teach an examination of centrosomes in cells where the p53 tumor suppressor gene is mutated (Abstract).

With regard to claim 17, Fukusawa teach an embodiment of claim 1, wherein the target is centrosomal (Abstract, Figures 1-2, where centrosomes were identified in mouse embryonic fibroblasts with wildtype and mutated p53 using an antibody to γ-tubulin).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to include the centrosomal target taught by Fukusawa into the method of in situ hybridization taught by Bresser. As noted by Fukusawa, the centrosomal target was crucial and in cells where p53 was mutated, "the presence of multiple centrosomes in the mitotic spindles had profound effects on chromosome segregation; the chromosomes did not partition during anaphase because they were captured by astral microtubules of one centrosome (or a few

centrosomes) localized outside of the poles". Fukusawa goes on to note that "when large numbers of centrosomes failed to localize at the poles in a bipolar fashion, the proper spindle apparatus did not form" (p. 1746, col. 1-2). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to include the centrosomal target taught by Fukusawa in order to determine the proper chromosomal segregation in a cellular target with a reasonable expectation for success.

Conclusion

16. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Lader (US Patent 6,204,375; March 2001) discloses a reagent for preserving and protecting the RNA content of tissue samples prior to RNA isolation. Ryan (US Patent 6,337,189; January 2002) teaches a system and composition for the stabilization of biological specimens which employs a fixative that comprises an organic solvent which includes alcohol.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephanie K. Mummert whose telephone number is 571-272-8503. The examiner can normally be reached on M-F, 8:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Stephanie K. Mummert

Stephanie K Mummert
Examiner
Art Unit 1637

SKM

JEFFREY FREDMAN
PRIMARY EXAMINER
4/25/06